JOURNAL OF BACTERIOLOGY, Mar. 1980, p. 1291-1297
0021-9193/80/03-1291/07$02.00/0

Vol. 141, No. 3

β-Alanine Synthesis in *Escherichia coli*

JOHN E. CRONAN, JR.

Department of Microbiology, University of Illinois, Urbana, Illinois 61801

The enzyme, aspartate 1-decarboxylase (L-aspartate 1-carboxy-lyase; EC 4.1.1.15), that catalyzes the reaction aspartate → β-alanine + CO_2 was found in extracts of *Escherichia coli. panD* mutants of *E. coli* are defective in β-alanine biosynthesis and lack aspartate 1-decarboxylase. Therefore, the enzyme functions in the biosynthesis of the β-alanine moiety of pantothenate. The genetic lesion in these mutants is closely linked to the other pantothenate (*pan*) loci of *E. coli* K-12.

D-Pantothenate, the condensation product of β-alanine and D-pantoic acid, is found in the 4'-phosphopantetheine moieties of coenzyme A (CoA) and of the acyl carrier protein of lipid synthesis (1, 6). Due to our interest in acyl carrier protein, we have attempted to alter the amount of the 4'-phosphopantetheine prosthetic group of this protein by manipulation of the specific precursors of the prosthetic group, namely β-alanine, pantoic acid, and pantothenate. In the course of these investigations, we realized that our knowledge of the pantothenate pathway was incomplete in that the pathway of β-alanine synthesis was not yet clear (3, 6).

The reactions leading to the formation of pantoic acid, the condensation of pantoate with β-alanine to form pantothenate, and the condensation of the pantothenate moiety with cysteine to form (ultimately) 4'-phosphopantetheine, are well established (6). However, the data on β-alanine synthesis are of a conflicting and indirect nature.

Two pathways of β-alanine synthesis have been proposed for *Escherichia coli*. The first pathway is the aspartate 1-decarboxylase pathway discussed in this paper. This pathway was proposed on the basis of the production of β-alanine by intact cells of *E. coli* incubated in the presence of aspartate (8, 35) and upon the reversal by β-alanine of growth inhibition by hydroxyaspartate (28). However, as pointed out by Boeker and Snell (3) and Brown (6), these data did not demonstrate that β-alanine was formed directly from aspartate.

The second pathway was proposed by Slotnick and Weinfeld (29, 30) from their finding that growth of a β-alanine auxotroph of *E. coli* was supported by dihydrouracil or β-ureidopropionate (*N*-carbamyl β-alanine). These workers suggested that the de novo synthesis of β-alanine proceeded from uracil through reduction to dihydrouracil and by hydrolysis first to β-ureidopropionate and then to CO_2, NH_3, and β-alanine.

In this paper, I demonstrate that the main pathway for β-alanine biosynthesis in *E. coli* is by the aspartate 1-decarboxylase. After the submission of this paper for publication, Williamson and Brown (39) reported the purification and properties of an aspartate 1-decarboxylase from *E. coli* W. The present paper has been revised to include a discussion of that work.

MATERIALS AND METHODS

Materials. Three lots of L-[*U*-^{14}C]aspartic acid were purchased from Amersham Corp. or New England Nuclear Corp. β-[1-^{14}C]alanine and β-[3-^{3}H]alanine were the products of New England Nuclear. Silica Gel G thin-layer plates (250 μm thick) were purchased from Analtech. The cellulose thin-layer plates were from Eastman Chemical Products, Inc. The amino acids, *N*-(2,4-dinitrophenol)-amino acids (DNP-amino acids), glutamic decarboxylase, and vitamins were from Sigma Chemical Co. Ketopantoic acid (14) and succinic dihydrazine (23) were synthesized by published procedures.

Preparation of extracts. The various bacterial strains listed in Table 1 were grown in a rich broth medium (7) (or in minimal medium as given) to late log phase at 37°C and collected by centrifugation. After the cells were washed with cold (4°C) distilled water, they were suspended in cold distilled water and incubated in ice for 20 to 30 min to discharge the cellular amino acid pools (4). After centrifugation the cells were suspended in 0.1 M potassium phosphate (pH 6.8) and disrupted in a French press at 18,000 lb/in^2. Large debris and unbroken cells were removed by centrifugation at 6,000 × *g* for 10 min. Protein concentrations were determined by the microbiuret reaction (20) by using bovine serum albumin as the standard.

Enzyme assays. Pantothenate synthetase was assayed as described by Miyatake et al. (19). Aspartate 1-decarboxylase was generally assayed by the conversion of ^{14}C-labeled aspartate to β-alanine as assayed by thin-layer chromatography. A standard reaction mixture contained L-[*U*-^{14}C]aspartate, 23 μM (216 mCi/mmol); potassium phosphate, 0.1 M (pH 6.8); and enzyme (0 to 90 μg of crude extract) in a final volume of 50 μl. The reaction mixtures (in polypropylene centrifuge tubes) were incubated at 37°C for 1 to 2 h.

After incubation, 0.2 ml of absolute ethanol and 5 μl of a mixture of 0.2 mM β-alanine–0.2 mM L-aspartate were added as carrier to each tube, and the resulting mixture was centrifuged to remove precipitated protein. The supernatant and a 100-μl wash (ethanol) of the pellet were combined in a second centrifuge tube and evaporated to dryness under N_2 at 80°C. The residue was dissolved in 50% ethanol and quantitatively spotted on a silica gel thin-layer plate. The plate was developed once or twice in absolute ethanol–28% ammonia (4:1, vol/vol) and then autoradiographed. The appropriate areas of the silica gel were then scraped from the plate into a vial containing 0.5 ml of water, and 3.5 ml of scintillant (PCS, Amersham) was added for scintillation counting.

Alternatively, the purified enzyme could be assayed by $^{14}CO_2$ release. The reaction was run in rubber septum-sealed vessels containing a disposable center well (Kontes). After incubation, 5 μl of glacial acetic acid was added to the reaction and 200 μl of Hyamine (New England Nuclear) was added to the center well. After the $^{14}CO_2$ had been collected (1 h at 37°C), the center well was placed into a scintillation vial containing 3 ml of PCS, and the $^{14}CO_2$ was quantitated by scintillation counting.

A third assay was sometimes used with the purified enzyme. After incubation, the reaction mixture was pipetted into a column (0.5 by 2.5 cm) of AG 1-X4 ion-exchange resin (chloride form) in a pipette plugged with glass wool. β-Alanine was eluted with two 0.5-ml washes with water and the water was counted in 3 ml of PCS. Aspartate remained bound to the column, but could be quantitatively eluted with 1 ml of 1 N HCl.

Control experiments using β-[1-^{14}C]alanine in the first and third assays and $^{14}CO_2$ in the second assay showed that recovery of the compounds was quantitative and that no degradation of β-alanine occurred in crude extracts. One unit of decarboxylase activity is defined as 1 pmol of product formed per min. The assay was linear with time for at least 3 h and with activity to >1 U per reaction in crude extracts and to >15 U per reaction with the purified enzyme.

TABLE 1. *Bacterial strains*

Strain	Relevant genotype	Source
E. coli[a]		
KL16	*pan*$^+$	K. B. Low (CGSG)
AB352	*pan*$^+$	E. Adelberg (CGSG)
AB353	*panD1*	E. Adelberg (CGSG)
AB354	*panD2*	E. Adelberg (CGSG)
AB355	*panD3*	E. Adelberg (CGSG)
CY262	*pan*$^+$	*pan*$^+$ transductant of AB354
CY263	*pan*$^+$	*pan*$^+$ revertant of AB355
AB2638	*panC5*	E. Adelberg (CGSG)
AT1371	*panC4*	A. L. Taylor (CGSG)
CS8AspTL	*aspA22*	Marcus and Halpern (18) (CGSG)
CY257	*panB6*	From strain Hfr 3000 YA139[b]
W	*pan*$^+$	C. Woolfolk
M99-1	*panC*	B. Davis (17)
M99-2	*panD*	B. Davis (17)
S. typhimurium LT2		
SA965	*pan*$^+$	K. Sanderson
pan-6	*panA6*	K. Sanderson (9)
pan-4	*panB4*	K. Sanderson (9)
pan-2	*panC2*	K. Sanderson (9)

[a] All strains are derivatives of *E. coli* K-12 except strains W, M99-1, and M99-2, which are *E. coli* W strains. Strains marked CGSG were from the Coli Genetic Stock Center, Yale University, New Haven, Conn.

[b] Strain CY257 is a *leu*$^+$ *panB6* derivative of strain PC0540 (CGSG 5407). Strain PC0540 was transduced first to *leu*$^+$ *ace*F with P1 phage grown on an *aceF* strain and then to *ace*$^+$ *panB* with phage grown on strain Hfr 3000 YA139.

Enzyme purification. An extract of strain AB352 was prepared as described above and centrifuged at 48,000 × *g* for 30 min. The resulting supernatant was heated in a 67°C water bath for 20 min, cooled in ice, and centrifuged at 35,000 × *g* for 30 min at 4°C. The supernatant (at 4°C) was fractionated with ammonium sulfate, and the precipitate formed from 42 to 60% of saturation was harvested by centrifugation, suspended in 0.1 M potassium phosphate (pH 6.8), and dialyzed against 200 volumes of the same buffer overnight at 4°C. The enzyme was stored at −20°C and retained full activity for at least 2 months. Pantothenate synthetase was purified as described previously (24).

Genetic methods. The genetic methods and the media used were described previously (7).

Chromatography. The solvent systems used in the identification of the product as β-alanine are listed by Niederwieser (21). For chromatography of the free amino acids on silica gel thin-layer plates, solvent systems 3, 5, 6, and 7 of Niederwieser (21) were used in various two-dimensional combinations. Either solvent system 7 (96% ethanol–28% ammonia, 70:30, vol/vol) or a modification of it (absolute ethanol–28% ammonia, 80:20, vol/vol) was used in one-dimensional chromatography. For chromatography on thin layers of cellulose (Eastman), the solvents used (in two-dimensional analysis) were first system 25 and then system 27.

The DNP-amino acids were synthesized essentially as described by Niederwieser (21) and extracted into ether. The DNP-amino acids were separated on thin layers of Silica Gel G in two dimensions by using various combinations of the solvent systems 50, 53, and 54 (21).

Radioactive amino acids were detected by autoradiography. Nonradioactive free amino acids were detected by ninhydrin, and the DNP-amino acids were detected by their characteristic color.

RESULTS

Identification of the reaction products. Two main products were formed by incubation of L-[*U*-^{14}C]aspartate with crude extracts of *E. coli* (Fig. 1). The product of higher chromatographic mobility is fumarate formed by the action of aspartase (13). The identification of this product as fumarate is based on cochromatography with authentic fumarate and the lack of

this product in a strain deficient in aspartase, strain CS8aspTL (18).

The product of lower mobility is β-alanine. The identification of the product as β-alanine is based on (i) Silica Gel G thin-layer chromatography in four different two-dimensional systems, (ii) on cellulose thin-layer chromatography in a two-dimensional system, and (iii) Silica Gel G thin-layer chromatography of its DNP derivative in three different two-dimensional solvent systems. In all cases, the radioactive spot exactly corresponded with that of β-alanine or DNP β-alanine added as an internal standard.

The production of β-alanine was confirmed by isotope dilution analysis. The mixture of products and substrate resulting from a standard reaction (10-fold increase in volume) was mixed with 1.2 g of authentic crystalline β-alanine, dissolved in water, and crystallized by addition of ethanol. After a second crystallization the specific activity of the crystals reached the value found through four subsequent recrystallizations (106.5 ± 8 cpm/mg).

A further confirmation of the identity of the product was the conversion of the β-alanine to pantothenate by pantothenate synthetase (D-pantoate:β-alanine ligase [AMP forming]; EC 6.3.2.1) (16, 19, 24). The conversion to pantothenate required D-pantoate, ATP, and Mg^{2+} and was confirmed by two-dimensional thin-layer chromatography (10).

It should be noted that no synthesis of L-alanine was seen, and therefore no decarboxylation occurred at the C-4 of aspartate. The absence of L-alanine was based on chromatographic evidence and on the lack of CO_2 production from L-[4-^{14}C]aspartate. This finding is consistent with the in vivo evidence of Roberts et al. (26), who reported that aspartate is not a precursor to L-alanine. Traces of other compounds were sometimes formed from L-[*U*-^{14}C]-aspartate in crude extracts which were tentatively identified as lysine, methionine, and threonine. These activities were lost upon prolonged dialysis or purification of crude extracts.

Partial purification and characterization of the decarboxylase. Aspartate 1-decarboxylase is a soluble enzyme. No activity was found in a washed membrane fraction. The enzyme was partially purified by heat treatment and ammonium sulfate fractionation. Over 94% of the initial activity was found in the 42 to 60% ammonium sulfate cut. The resulting enzyme preparations were about 40-fold purified over the crude extract with a yield of 140%. The increase in total activity is attributed to the heat inactivation of the competing aspartase reaction. The purified enzyme preparations contained only traces of aspartase. Similar heat and ammonium sulfate steps were used by Williamson and Brown (39).

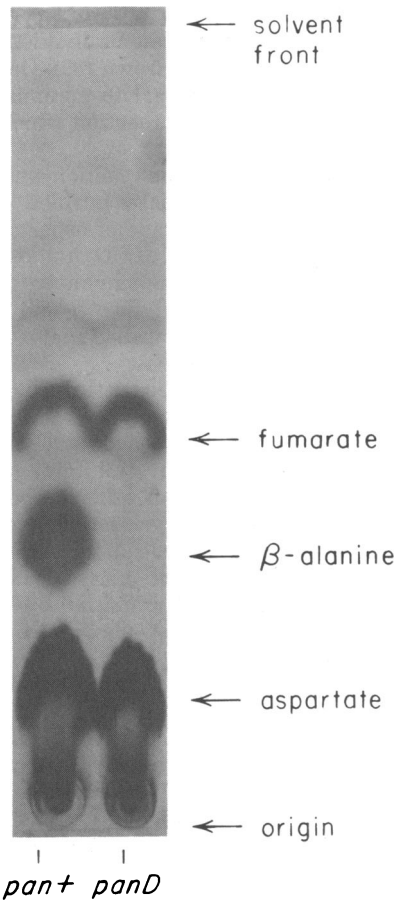

FIG. 1. *Autoradiogram of the products of an incubation of* L-*[U-*^{14}*C]aspartate with 90 μg of protein from strain CY362 (pan*$^{+}$*) or strain AB354 (panD) as described in the text. The silica gel thin-layer plate was developed in absolute ethanol–28% NH_4OH (4:1, vol/vol).*

The pH optimum of the aspartate 1-decarboxylase activity was about pH 7.5. The reaction rates at the pH values of 6.8 and 8.0 were about 60% of the pH 7.5 rate. A similar broad pH optimum (pH 6.5 to 7.5) was reported by Williamson and Brown (39).

The Michaelis constant of the decarboxylase for aspartate was about 80 μM. Similar values were given by crude extracts and by the partially purified enzyme preparation. Williamson and Brown (39) reported values of 160 and 300 μM, respectively, for the major and minor forms found in *E. coli* W. The specific activities reported for crude extracts differ between the two laboratories, but this is due to differing assay conditions. When corrected to the same sub-

strate concentration, assay pH, and assay temperature, our results are essentially identical to those of Williamson and Brown (39). Our assay was run at a suboptimal pH to minimize competition by the aspartase reaction which has a higher pH optimum (13).

The stoichiometry of the reaction was determined by using the partially purified enzyme (Table 2). For each molecule of aspartate consumed, one molecule each of CO_2 and β-alanine were formed. The same stoichiometry was reported by Williamson and Brown (39) for the reaction products of the purified major form of the decarboxylase.

Evidence for an essential carbonyl group. Aspartate 1-decarboxylase was inhibited by a variety of carbonyl reagents. Hydroxylamine was the most effective inhibitor (81% inhibition at 60 μM), but all other carbonyl reagents tested ($NaBH_4$, D-cycloserine, hydrazine, semicarbazine, and succinic dehydrazine) also inhibited aspartate 1-decarboxylase. Williamson and Brown (39) reported inhibition by hydroxylamine and $NaBH_4$ of the major form of the decarboxylase.

Williamson and Brown (39) demonstrated that the essential carbonyl of the major form of the decarboxylase is that of a pyruvoyl residue. However, the prosthetic group of the minor form of the decarboxylase was not studied. I found that about 10% of the original activity of ammonium sulfate-purified decarboxylase preparations (reportedly [39] a mixture of the two forms) was inactivated by treatment with cycloserine (12), but this could be restored by incubation with pyridoxal phosphate. It seems possible therefore that the essential carbonyl group of the minor decarboxylase form is that of pyridoxal phosphate.

Inhibitor and substrate specificity. In agreement with Williamson and Brown (39), I found that D-aspartate was neither a substrate nor an inhibitor of the decarboxylase. We further agree that D-serine is a competitive inhibitor and is therefore a plausible target for the known inhibition of pantothenate synthesis by D-serine (17).

TABLE 2. *Stoichiometry of aspartate 1-decarboxylase reaction*[a]

Expt	CO_2 released (nmol)	β-Alanine formed (nmol)	L-Aspartate consumed (nmol)
1	10.5	10.4	9.9
2	5.8	8.4	5.3

[a] A standard reaction was run with L-[*U*-^{14}C]aspartic acid by using 65 μg of ammonium sulfate-purified enzyme in experiment 1 and half that amount in experiment 2. After incubation, CO_2 was collected as described in the text, and then carrier β-alanine and L-aspartic acid were added. β-[3-^{3}H]alanine (1 μCi, 37.5 Ci/mmol) was added to monitor the recovery of β-alanine. The L-[*U*-^{14}C]aspartate was uniformly labeled as determined by ninhydrin treatment and by treatment with a glutamate-5 (aspartate-4) decarboxylase preparation from *Clostridium welchii* (11).

Physiological function of aspartate 1-decarboxylase. Pantothenate auxotrophs of *E. coli* and *Salmonella typhimurium* are of three types (Fig. 2). *panC* mutants respond only to pantothenate, whereas *panB* mutants also respond to pantoic or ketopantoic acids (or their lactones) and *panD* mutants respond to β-alanine as well as pantothenate (9, 17). *panD* mutants, therefore, seem specifically defective in the synthesis of β-alanine and thus should be deficient in aspartate 1-decarboxylase. This is the case. Four different *panD* mutants were found to contain no detectable decarboxylase activity (Table 3). The most thoroughly characterized mutant, strain AB354, contained <0.3% of the enzyme level found in the wild-type strain. The other strains, AB353, AB355, and M99-2, contained less than 2% of the normal activity (Table 3). Mixtures of the inactive extracts with active extracts gave additive activities, indicating the lack of an inhibitor in the *panD* extracts. A *pan*$^+$ revertant of strain AB355 regained normal decarboxylase activity, and a *pan*$^+$ transductant of strain AB354 (the P1 phage were grown on strain AB352) also contained a normal level of decarboxylase (Table 3). These results indicate that a single mutation is responsible for the lack of decarboxylase in *panD* auxotrophs and therefore demonstrate that the major pathway of β-alanine synthesis in *E. coli* is by the α-decarboxylation of L-aspartate.

S. typhimurium LT2 strains have aspartate 1-decarboxylase levels similar to those of *E. coli* (Table 3). The enzyme levels of the *panD* mutants of *S. typhimurium* (22) were not determined because these strains could not be obtained. It should be noted that Williamson and Brown (39) found that *E. coli* M99-2 had only about 10% of the decarboxylase activity found in *E. coli* W.

The level of aspartate 1-decarboxylase activity in strain AB352 was unaffected by supplementation of a defined growth medium with β-alanine or a mixture of β-alanine, pantoic acid, and pantothenate (Table 3). Therefore, the decarboxylase, like other pantothenate biosynthetic enzymes (32), is not repressed by end products. *panB* and *panC* auxotrophs of *E. coli* had normal decarboxylase levels (Table 3).

Genetic mapping of the *panD* locus. The *pan* auxotrophs of *E. coli* K-12 had not been

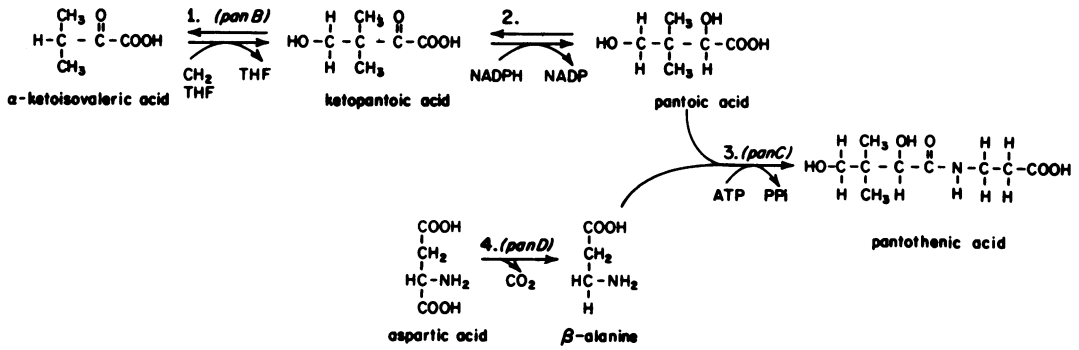

FIG. 2. *Pantothenate synthesis in E. coli. The reaction steps are numbered, and the gene loci believed to code for the enzymes catalyzing the steps are given in parentheses. Step 1 is catalyzed by ketopantoate hydroxymethyl transferase; step 2 could occur on the free acid (as drawn) by using ketopantoate reductase or as the lactone with ketopantoyl lactone reductase. Step 3 is catalyzed by pantothenate synthetase, and step 4 is the aspartate 1-decarboxylase reaction. The systematic name for ketopantoic acid is 2-keto-4-hydroxy-3,3-dimethylbutyric acid. See the text for discussion of this scheme.*

classified as thoroughly as the *pan* auxotrophs of *S. typhimurium* and *E. coli* W. We found that the Coli Genetic Stock Center collection consists of one *panB* auxotroph (responding to pantoic acid and ketopantoic acid) and two *panC* auxotrophs in addition to the three *panD* auxotrophs. The *E. coli* K-12 strains were classified by nutritional tests and by cross-feeding experiments with the *E. coli* W and *S. typhimurium* auxotrophs.

Both the *panB* and *panC* loci were known to map between the *tonA* and *dapD* loci (min 3) on the *E. coli* genetic map (2). The *panA*, *panB*, and *panC* loci of *S. typhimurium* map in a cluster in the analogous place on the map of that organism (9, 27). However, because the *panD* locus of *S. typhimurium* has recently been reported to map at min 89 (22), we mapped the *panD* locus of *E. coli* K-12.

Conjugational crosses of phenocopies of the three *panD* strains with HfrH and HfrC strains indicated that the *panD* locus was closely linked to the *panB* and *panC* loci. This was confirmed by P1 cotransduction of the *panD*, *panB*, and *panC* loci. P1 phage grown on strain AB354 (*panD*) was used to transduce strain CY257 (*panB*) to *panB*$^+$ in the presence of β-alanine. All 73 transductants were *pan* strains whose requirement was met by β-alanine. An analogous experiment gave 95% cotransduction of *panB* with *panC*. Conjugational crosses of the Hfr *panD* strains AB353, AB354, and AB355 with the *panC* strain AT1371 all gave a >98.5% linkage between *panD* and *panC*, whereas linkage of *panD* with the *leu* locus at min 1.5 was only about 60%. The results therefore indicate that the *panB*, *panC*, and *panD* loci are tightly linked and possibly comprise an operon.

TABLE 3. *Aspartate 1-decarboxylase levels in various strains*

Strain	Characteristics	Sp act[c] (U/mg of protein)
KL16	*pan*$^+$	2.9
AB352	*pan*$^+$	3.1
AB354	*panD*	<0.005
AB353	*panD*	<0.08
AB355	*panD*	<0.06
CY266	*pan*$^+$ transductant of AB354	3.2
CY267	*pan*$^+$ revertant of AB355	4.8
AB1371	*panC*	3.4
CY257	*panB*	5.5
AB352	Defined medium[a]	3.6
AB352	Defined medium + β-alanine	3.8
AB352	Defined medium + β-alanine, pantoic acid, and pantothenate	2.7
AB352	Minimal medium[b]	3.3
E. coli W	*pan*$^+$	4.2
M99-1	*panC* of *E. coli* W	1.2
M99-2	*panD* of *E. coli* W	<0.05
SA965	*pan*$^+$ of *S. typhimurium* LT2	4.8

[a] The defined medium consisted of medium E (36) supplemented with glucose, 0.4%; adenine, 20 μg/ml; thiamine, 1 μg/ml; and vitamin-free casein hydrolysate, 0.2%. Pantothenate, pantoic acid, and β-alanine, when present, were added at 0.01%. All other strains were grown in R broth (7).

[b] Minimal medium is the defined medium with the casein hydrolysate replaced with 20 μg each of threonine and leucine per ml.

[c] Specific activity of crude extracts; the assay was performed at a suboptimal pH and aspartate concentration (see text). Units are picomoles minute^{-1} determined at 37°C.

Lack of response to dihydrouracil. In conflict with the previous results (29, 30), we find that none of the *E. coli panD* auxotrophs respond to dihydrouracil. This has also been observed for the *S. typhimurium* strains (22). The *E. coli* strains do respond to β-ureidopropionate (*N*-carbamyl β-alanine), but this can be attributed to the spontaneous hydrolysis of the carbamyl group in the growth medium.

DISCUSSION

The results presented in this paper and that of Williamson and Brown (39) demonstrate that the synthesis of β-alanine in *E. coli* proceeds by α-decarboxylation of L-aspartate. *panD* mutants are deficient in aspartate 1-decarboxylase, and the enzyme defect seems due to a single mutational event. These data, coupled with the fact that dihydrouracil does not support the growth of the *E. coli* and *S. typhimurium panD* strains, indicate that the purine degradation pathway proposed by Slotnick and Weinfeld (29, 30) can be discarded as a major pathway of β-alanine synthesis.

Aspartate 1-decarboxylase could be a rate-limiting step in the synthesis of pantothenate. The activity of the enzyme should be maximal in vivo because the intracellular level of pyruvate (400 μM) is fivefold greater than the Michaelis constant of the decarboxylase for aspartate (15). Under optimal conditions (pH 7.5, saturating aspartate), a crude extract of 10^9 *E. coli* cells has sufficient decarboxylase activity to form about 0.8 to 1 μmol of β-alanine per h at 37°C. This is quite similar to the amount of β-alanine needed for 10^9 cells to double in mass (0.7 to 0.9 μmol) (34).

These considerations suggest that the formation of β-alanine could be the rate-limiting step in the formation of pantothenate. Powers and Snell (25) have suggested on the basis of end product inhibition experiments that ketopantoic hydroxymethyl transferase activity could also be limiting, whereas pantothenate synthetase activity seems to be present in a large excess (16, 19, 24). The rate-limiting step in pantothenate synthesis is probably also the rate-limiting step in CoA synthesis because supplementation of *E. coli* growth media with pantothenate increases the intracellular CoA pool by 10- to 20-fold (1, 5).

The present work, when coupled with recent biochemical data, gives a coherent picture of the pantothenate biosynthetic pathway in *E. coli* (Fig. 2). Apparently the same pathway functions in *S. typhimurium*. However, few of the genetic and biochemical data on this latter organism have been published. Most of the results are available only in summary form (9).

Ketopantoic acid, the first intermediate in the pantoic acid arm of the pantothenate pathway (Fig. 2), is formed from α-ketoisovaleric acid and formyl tetrahydrofolate by the enzyme ketopantoate hydroxymethyl transferase (25, 32). This enzyme is probably coded by the *panB* gene because a *panB* mutant of *E. coli* W lacks this enzyme (32). The ketopantoic acid is then reduced to pantoic acid either directly or after lactonization to ketopantoyl lactone (37, 38). Reductases for both the acid and the lactone have been demonstrated in *E. coli* and, furthermore, two forms of the ketopantoyl lactone reductase have been reported (37, 38). This multiplicity of enzymes may explain why no *pan* mutants able to use pantoic acid, but not ketopantoic acid, have been isolated.

The pantoic acid formed by reduction is then condensed with β-alanine to form pantothenate (16, 19, 24). The condensing enzyme, pantothenate synthetase, appears to be the product of the *panC* gene because a *panC* mutant of *E. coli* W lacks this enzyme (16). The genetic and enzymological studies are therefore in good agreement except for the *panA* mutant of *S. typhimurium* (9) (no *panA* mutants are known in *E. coli*).

The *panA* mutant responds to α-ketoisovaleric acid and valine as well as ketopantoic acid, pantoic acid, and pantothenate (9). Demerec and co-workers, therefore, proposed that this mutant is defective in the synthesis of α-ketoisovaleric acid (9). This proposal does not seem likely, as such a block would engender requirements for valine and leucine as well as pantothenate (33). It seems more likely that *panA* mutants produce an altered ketopantoate hydroxymethyl transferase with a decreased affinity for α-ketoisovalerate and thus the *panA* mutant might be a type of *panB* auxotroph. Studies to test this hypothesis are in progress.

Williamson and Brown (39) reported two distinct forms of aspartate 1-decarboxylase that differed greatly in both size and charge. However, *panD* mutants lacked both forms (Table 3; 39), and thus the two forms are related. Perhaps they share a common subunit coded by the *panD* gene. The major form of decarboxylase appears to be composed of three dissimilar subunits (39), and the large size of the minor component suggests a subunit structure.

Circumstantial evidence presented in this paper suggests that the minor form may be a pyridoxal phosphate enzyme. It would be most interesting if *E. coli* does contain two enzymes with different prosthetic groups, particularly because the relative amounts of the two decarboxylase forms depend on the composition of the medium in which the cells are cultured (39).

ACKNOWLEDGMENTS

This work was supported by Public Health Service grant AI 15650 from the National Institute of Allergy and Infectious Diseases.

I thank Barbara Bachmann for her advice and the gifts of strains.

LITERATURE CITED

1. **Alberts, A. W., and P. R. Vagelos.** 1966. Acyl carrier protein. VIII. Studies of acyl carrier protein and coenzyme A in *Escherichia coli* pantothenate or β-alanine auxotrophs. J. Biol. Chem. **241**:5201–5204.
2. **Bachmann, B. J., K. B. Low, and A. L. Taylor.** 1976. Recalibrated linkage map of *Escherichia coli* K-12. Bacteriol. Rev. **40**:116–167.
3. **Boeker, E. A., and E. S. Snell.** 1972. Amino acid decarboxylases, p. 217–253. *In* P. Boyer (ed.), The enzymes, vol. VI, Academic Press Inc., New York.
4. **Britten, R. G., and F. T. McClure.** 1962. The amino acid pool in *Escherichia coli*. Bacteriol. Rev. **26**:292–335.
5. **Brown, G. M.** 1959. The metabolism of pantothenic acid. J. Biol. Chem. **234**:370–380.
6. **Brown, G. M.** 1970. Biosynthesis of pantothenic acid and coenzyme A, p. 73–80. *In* M. Florkin and E. H. Stotz (ed.), Comprehensive biochemistry, vol. 21. Elsevier, Amsterdam.
7. **Cronan, J. E., Jr., D. F. Silbert, and D. L. Wulff.** 1972. Mapping of the *fabA* locus for unsaturated fatty acid synthesis in *Escherichia coli*. J. Bacteriol. **112**:206–211.
8. **David, W. E., and H. C. Lichstein.** 1950. Aspartic acid decarboxylase in bacteria. Proc. Soc. Exp. Biol. Med. **73**:216–218.
9. **Demerec, M. E., E. L. Lahr, E. Balbinder, T. Miyake, C. Mack, D. Mackay, and J. Ishidu.** 1959. Bacterial genetics. Carnegie Inst. Washington Yearb. **58**:433–440.
10. **Frank, M. J., P. G. Trager, J. B. Johnson, and S. H. Rubin.** 1973. Characterization of crystalline calcium pantothenate by physiochemical assay methods. J. Pharm. Sci. **62**:108–111.
11. **Greenberg, D. M., and M. Rothstein.** 1957. Methods for chemical synthesis, isolation, and degradation of labeled compounds. Methods Enzymol. **4**:652–731.
12. **Klosterman, H. J.** 1979. Vitamin B_6 antagonists of natural origin. Methods Enzymol. **62**:483–495.
13. **Liess, K., D. Mecke, and H. Holzer.** 1966. Zur regulation du synthese von aspartase in *E. coli*. Biochem. Z. **346**: 244–251.
14. **Lipton, A. H., and F. M. Strong.** 1949. Synthesis of compounds related to pantothenic acid. J. Am. Chem. Soc. **71**:2364–2367.
15. **Lowry, O. H., J. Carter, J. B. Ward, and L. Glaser.** 1971. The effect of carbon and nitrogen sources on the level of metabolic intermediates in *Escherichia coli*. J. Biol. Chem. **246**:6511–6521.
16. **Mass, W. K.** 1952. Pantothenate studies. III. Description of the extracted pantothenate-synthesizing enzyme of *Escherichia coli*. J. Biol. Chem. **198**:23–32.
17. **Mass, W. K., and B. D. Davis.** 1950. Pantothenate studies. I. Interference by D-serine and L-aspartic acid with pantothenate synthesis in *Escherichia coli*. J. Bacteriol **60**:733–745.
18. **Marcus, M., and Y. S. Halpern.** 1969. Mapping of the aspartase gene in *Escherichia coli* K-12. Isr. J. Med. Sci. **5**:413–415.
19. **Miyatake, K., Y. Nakano, and S. Kitaoka.** 1979. Pantothenate synthetase from *Escherichia coli*. Methods Enzymol. **62**:215–219.
20. **Munkres, K. D., and F. M. Richards.** 1965. The purification and properties of *Neurospora* maleate dehydrogenase. Arch. Biochem. Biophys. **109**:466–479.
21. **Niederwieser, A.** 1972. Thin-layer chromatography of amino acids and derivatives. Methods Enzymol. **25**:60–99.
22. **Ortega, M. V., A. Cardenas, and D. Ubiera.** 1975. *panD*, a new chromosomal locus of *Salmonella typhimurium* for the biosynthesis of β-alanine. Mol. Gen. Genet. **140**:159–164.
23. **Parikh, I., S. March, and P. Cuatrecasas.** 1974. Topics in the methodology of substitution reaction with agarose. Methods Enzymol. **34B**:77–102.
24. **Pfleiderer, G., A. Kreiling, and T. Wieland.** 1960. Über Pantothen-säuresynthetase aus *E. coli*. Biochem. Z. **333**:302–307.
25. **Powers, S. G., and E. E. Snell.** 1976. Ketopantoate hydroxymethyl-transferase. II. Physical, catalytic, and regulatory properties. J. Biol. Chem. **251**:3786–3793.
26. **Roberts, R. B., P. H. Abelson, P. B. Cowie, E. T. Bolton, and R. J. Britten.** 1963. Studies of biosynthesis in *Escherichia coli*. Carnegie Inst. Washington. Publ. **607**:247–272.
27. **Sanderson, K. E., and P. E. Hartman.** 1978. Linkage map of *Salmonella typhimurium*, edition V. Microbiol. Rev. **42**:471–519.
28. **Shive, W., and J. Macow.** 1946. Biochemical transformation as determined by competitive analogue—metabolite growth inhibition. I. Some transformation involving aspartic acid. J. Biol. Chem. **162**:451–462.
29. **Slotnick, I. J.** 1956. Dihydrouracil as a growth factor for a mutant strain of *Escherichia coli*. J. Bacteriol. **72**: 276–277.
30. **Slotnick, I. J., and H. Weinfeld.** 1957. Dihydrouracil as a growth factor for mutant strains of *Escherichia coli*. J. Bacteriol. **74**:122–125.
31. **Snell, E. E.** 1977. Pyruvate-containing enzymes. Trends Biochem. Sci. **2**:131–135.
32. **Teller, J. H., S. G. Powers, and E. E. Snell.** 1976. Ketopantoate hydroxymethyltransferase. I. Purification and role in pantothenate biosynthesis. J. Biol. Chem. **251**:3780–3785.
33. **Umbarger, H. E.** 1975. Regulation of amino acid biosynthesis in microorganisms, p. 1–56. *In* H. R. V. Cunstein (ed.), MTP international review of science vol. 7. Butterworths, London.
34. **Van den Bosch, H., J. R. Williamson, and P. R. Vagelos.** 1970. Localization of acyl carrier protein in *Escherichia coli*. Nature (London) **228**:338–341.
35. **Virtanen, A. I., and T. Laine.** 1937. The decarboxylation of D-lysine and L-aspartic acid. Enzymologia **8**:266–270.
36. **Vogel, H. J., and D. M. Bonner.** 1956. Acetylornithinase of *Escherichia coli*: partial purification and some properties. J. Biol. Chem. **218**:97–106.
37. **Wilken, D. R., H. L. King, and R. E. Dyar.** 1975. Ketopantoic acid and ketopantoyl lactone reductases. J. Biol. Chem. **250**:2311–2314.
38. **Wilken, D. R., H. L. King, and R. E. Dyar.** 1979. Ketopantoyl lactone reductases. Methods Enzymol. **62**: 209–215.
39. **Williamson, J. M., and G. M. Brown.** 1979. Purification and properties of L-aspartate-α-decarboxylase, an enzyme that catalyses the formation of β-alanine in *Escherichia coli*. J. Biol. Chem. **254**:8074–8082.